PATTERNS OF CHANGES IN THE CELL MEDIATED IMMUNITY IN PATIENTS RECEIVING BLOOD TRANSFUSIONS

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ARVIND KUMAR VAISH

Department of Surgery,
M.L.B. Medical College,
Jhansi.

C E R T I F I C A T E

This is to certify that the work entitled as "PATTERNS OF CHANGES IN THE CELL MEDIATED IMMUNITY IN PATIENTS RECEIVING BLOOD TRANSFUSIONS", which is being submitted as THESIS for M.S.(General Surgery) examination, 1989 of Bundelkhand University, Jhansi, has been carried out by DR. ARVIND KUMAR VAISH, himself in this department.

He has put in the necessary stay in the department as required by the regulations of Bundelkhand University.

Dated : 31st August, 1988.

S L Agarwal

(S. L. Agarwal)
M.S., F.R.C.S.,
Professor and Head,
Department of Surgery,
M.L.B. Medical College,
JHANSI.

Department of Surgery,
M.L.B. Medical College,
Jhansi.

C E R T I F I C A T E

This is to certify that the present work entitled "PATTERNS OF CHANGES IN THE CELL MEDIATED IMMUNITY IN PATIENTS RECEIVING BLOOD TRANSFUSIONS", which is being submitted as THESIS for M.S.(General Surgery) examination, 1989, has been carried out by DR. ARVIND KUMAR VAISH, under my constant supervision and guidance. The results and observations were checked and verified by me from time to time. The techniques embodied in this work were undertaken by the candidate himself.

This work fulfils the basic ordinance governing the submission of thesis laid down by Bundelkhand University.

Dated : 3rd August, 1989.

(Rajeev Sinha)
M.S.,
Lecturer,
Department of Surgery,
M.L.B. Medical College,
JHANSI.

(CHIEF GUIDE)

DEPARTMENT OF PATHOLOGY,
M.L.B. MEDICAL COLLEGE,
JHANSI.

CERTIFICATE

This is to certify that MR. ARVIND KUMAR VAISH has worked on "PATTERNS OF CHANGES IN THE CELL MEDIATED IMMUNITY IN PATIENTS RECEIVING BLOOD TRANSFUSIONS" under my guidance and supervision.

His results and observations have been checked and verified by me from time to time.

Dated : 21.8.88

(Ratna Saxena)
M.D.,
Lecturer,
Department of Pathology,
M.L.B. Medical College,
JHANSI.

(CO-GUIDE)

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(Arvind Kumar Vaish)

Arvind Vaish

C O N T E N T S

INTRODUCTION

INTRODUCTION

With the gradual passage of time since the familiarisation with blood transfusion, during the Second world war, more and more complications and beneficial effects of blood transfusion were recognised. While the routine complications were recognised quite early, it wasn't until the 1970's that the immunodepressive effect of blood transfusion was recognised.

This immunodepressive effect was perceived for the first time in patients receiving kidney grafts. It was seen that patients receiving pretransplant blood transfusions often showed better graft survival as compared to patients who did not receive blood transfusion. With the gradual passage of time more and more workers seemed to agree with these findings.

The next mile stone was crossed during the last five years, when it was shown that perioperative blood transfusions in patients undergoing surgical treatment for solid malignancies showed an increase in recurrence rate and a poorer survival rate. This was seen in a number of malignancies as carcinoma of the colon, breast, urogenial malignancies and malignancies of the lung.

The depression of immunological status is also reflected by the fact that blood transfusion leads to an increased susceptibility to infectious complications.

In the last few years various studies have been published on the immunodepressive effect of blood transfusions either of single unit or multiple units. Various immunological parameters have been studied by different workers in both animal models and in patients receiving blood transfusions, with the same inference of post transfusion cellular immunodepression.

The aim of the study is to determine the change in the cell mediated immunity after transfusion using lymphocyte count, T cell count and PHA skin reactivity tests as parameters.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Since the 1970's the immunomodulatory effect of blood transfusion has been known. Since that period, various studies have been carried out to show the immunodepressive action and to ascertain the precise mechanism of immunodepression. There are various indirect and direct evidences of this immunodepressive effect after single or multiple blood transfusions. These evidences were for the first time gathered from patients receiving blood transfusions during treatment for malignancy and in patients receiving kidney grafts.

INDIRECT EVIDENCES OF IMMUNODEPRESSIVE ACTION OF BLOOD TRANSFUSION

1. Effect of blood transfusion on graft survival

Historically, pretransplant blood transfusions were initially considered detrimental to graft survival since they were associated with recipient sensitization and thus increased the risk of hyperacute rejection.

But studies in the 1960's showed similar or increased graft survival in patients who had received many transfusions before transplantation compared to those who had received few (Dausset et al, 1967 and Morris et al, 1968). Amos et al (1963) had shown that blood transfusions can produce individual specific allograft sensitivity in normal human recipients with accelerated rejection of skin grafts obtained from the blood donors.

Marquet et al (1971) reported a specific inhibition of organ allograft rejection phenomena by donor blood transfusion in rats. They observed that a single intravenous injection of as little as 0.05 ml fresh donor blood given between one week and two months prior to transplantation increased kidney graft survival from 12 to 100 days. Blood given within a week of the transplant had a much less pronounced effect. These results were confirmed and extended by Fabre and Morris (1972). Abouna et al (1977) demonstrated that recipient treatment with whole blood transfusions from multiple donors is associated with a significant prolongation of renal graft survival in dogs. Similarly in the rhesus monkey Van Es et al (1977) have described prolongation of kidney allograft survival by one and more pronounced by five transfusions of 20 ml each. On the other hand Smith and Nyburg (1979) studied the effect of multiple blood transfusions on kidney transplant survival, in baboons and found no increase of graft survival in transfused animals.

The first evidence of effect of blood transfusions in human renal transplantation was supplied by Opelz et al, who published a retrospective study in man in 1973. They showed that recipient of cadaver kidney grafts who had not been transfused ptior to transplantation had a significantly lower graft

survival rate than transfused recipients. The same group have further substantiated their results in several reports (Opelz et al, 1974 and 76). Festenstein et al (1976) observed a similar effect and demonstrated marked improvement in best HLA matched groups. A number of later reports demonstrated improved cadaveric graft survival in transfused patients (Solheim et al, 1977; Fuller et al, 1977; Persijn et al, 1977; Sirchia et al, 1979 and Von Reed, 1979). In contrast to the above evidences, there are some indications from both clinical and experimental work that pre transplant blood transfusion may on occasions be harmful and lead to accelerated rejection (Balner et al, 1978 and Wood et al, 1978).

The mechanisms of the beneficial effect of the blood transfusion have been variously hypothesised. Thus blood transfusions may exert their beneficial effect by the induction of non specific T and Non T suppressor cells that dampen the host immune response (Von Reed et al, 1978; Smith et al, 1981 and Keown et al, 1979). Kerman et al (1980) proposed that the beneficial effect of pretransplant blood transfusions on graft survival may be due to a direct effect of blood transfusion on immunocompetence. Patients receiving more than 5 blood transfusions display both, a weak immune responder status and increased suppressor cell function compared to patients receiving few or no transfusions. Klein Munn et al (1983) demonstrated

that blood transfusions induce in vivo, the generation of suppressor cells that are active towards alloantigens. Rejection of patients who were antibody formers, following transfusions, was claimed as a beneficial effect of blood transfusion on graft survival by Solheim (1979). Fahrnam et al (1983) support the above concept and show no effect on T cell reactivity following five planned transfusions and because fewer patients with antibodies received grafts as compared to patients without antibodies, blood transfusions seem to have led to a selection effect. Ring and Wigzell (1977) proposed that blood transfusion induced immunological unresponsiveness can be due to anti-idiotypic antibodies against T-cell antigen specific receptor. The above concept was further supported by Pagnilli et al (1982) and Singhal et al (1982). Chia et al (1982) who also suggested that Fab. Antibodies, and possibly anti IgG antibodies while Macleod et al(1983), claimed Fc receptors blocking antibodies were the cause of the enhancing effect of blood transfusions on graft survival.

2. <u>Effect of blood transfusion in malignancy</u>

Everson and Cole (1976) reviewed 176 well documented cases of spontaneous remission of cancer and suggested that blood transfusion was the trigger for the remission in some cases, particularly of

malignant melanoma. On the other hand Israel et al (1976) and others have claimed that removing plasma from metastatic cancer may induce remission.

Francis et al (1981) showed experimentally in female rats that the tumour growth increased after allogenic blood transfusion and supported the concept of non-specific immunosuppression after blood transfusion.

The first report of an adverse effect of blood transfusion, on survival comes from Burrows and Tarter (1982) who looked retrospectively at 122 patients who had undergone curative operations for colo-rectal cancers. Those who had not received a blood transfusion before, during or after their operation, survived longer without tumour recurrence. Since then other retrospective studies on colorectal cancer have confirmed the original observations (Foster et al, 1985 and Blumberg et al, 1985). But there are some reports to the contrary (Ota et al; Blair et al; Francis et al, 1985), which do not support the above findings.

The adverse effect of transfusions have also been reported for carcinoma of the breast (Tartter et al, 1983), Lung (Tartter et al, 1984 and Hyman et al, 1985), Kidney (Moffat et al, 1985), uterine cervix Blumberg et al, 1985) and for soft tissue sarcomas (Rosenberg et al, 1985). Foster et al (1984) examined 226 patients with breast cancer and followed them and

found no change in the survival rate of cancer patients receiving blood transfusions.

An explanation for the increase in growth or increase recurrence rate of tumour after transfusions can be the immunomodulatory effect of the blood transfusions possibly decreasing the immunoresponsiveness of the host to a tumour (Gantt, 1981). Alternative explanation is blood loss. The need of blood transfusions due to blood loss might be an indicator of cancer, that require greater degree of manipulation during resection which might conceivably be related to a greater degree of dissemination during operation. This explanation would be in keeping with the Turnbull's no touch technique (1967). Further the malignant growth which results in greater pre-operative and operative blood loss are biologically more aggressive and transfusion need may be a marker of worse prognosis (Foster et al, 1985).

DIRECT EVIDENCES OF IMMUNOMODULATORY EFFECT OF BLOOD TRANSFUSIONS

According to Schechter et al (1972) administration of even a small amount of blood causes a definite immunologic stimulation of the recipient. This conclusion was based on his study on post transfusion blood lymphocytes. He measured the lymphocyte H_3 thymidine uptake and counted atypical lymphocytes.

The patients transfused with fresh or stored blood had significantly greater average of H_3 thymidine uptake. This rise was seen only after the third day and the maximum uptake occured on the sixth or the seventh day after transfusion. Incorporation began to decline in the second week and returned to pre transfusion values by the third week. The rise in atypical lymphocytes was eight times more than the pre transfusion level in the transfused subject.

In the past few years various studies have been published on the immunodepressive effect of blood transfusions. Impaired cellmediated immunity following blood transfusions was observed by several authors (Fisher et al. 1980; Lenhard et al. 1982 and Karman,1982). Others did find significant changes only in multi-transfused patients (Fehrman et al. 1981; and Jeannet et al. 1982).

Various immunological parameters were studied by different workers in both animal models and in patients, receiving blood transfusions.

A suppressive effect of transfusion on cellular immunity as measured by PHA induced lymphocyte response was found by Berleffa and Marquet (1981) in rhesus monkeys. The depressed lymphocyte reactivity to the recal antigen PPD, and to the plant mitogen PHA and the raised inhibitory activity of plasma was noted by Francis et al in 1981, after allogenic blood transfusions

in female rats, and concluded that non specific immunosuppression resulted from blood transfusions.

The total numbers of lymphocytes usually dropped sharply during the first two days after transfusion. This drop occurs almost invariably in surgical patients. But by the seventh post operative day numbers of lymphocytes returned to pre-operative level (Schechter et al, 1972).

Lymphocyte response to an antigen Cocktail (Ag-C, Behringwerke Marburg; Containing PPD, tetanus toxoid, streptolysin, mumps, and vaccinia antigen) was measured by Lenhard et al (1982) and found that after transfusion, lymphocyte response to Ag-C was clearly suppressed to 56% of pre transfusion level within the first week and again nearly reached to pretransfusion values after 3 weeks (Lenhard et al, 1982).

In the post-transfusion period a transient decrease in the T cells was specifically observed by Lenhard et al (1982). According to Herman et al (1982) and 1983) blood transfusion caused transient immune changes with decrease in active T-RFC or spontaneous blastogenesis with increase percentage of T suppressor cells (OKT8 T cells) during a 3 month interval and strong suppressor cell function in vitro as measured by third party mixed lymphocyte culture.

Fehrman et al (1983) studied MLC reactivity and PHA stimulation tests for lymphocyte function in

non uremic patients receiving multiple blood transfusions. The results showed low MLC reactivity and low PHA responses in transfused group. The conclusions was that transfused patients have poor immunological responsiveness whether they are uremic or not.

Van Reed and Reimer (1978) suggest that post transfusion immunodepression is due to the induction of suppressor cells. Kletzmann et al (1983) suggest that blood transfusion induce, in vivo, the generation of suppressor cells that are active towards the alloantigen.

According to Smith et al (1981) a single transfusion of 2 units of red blood cells in renal dialysis patients produces a significant effect on suppressor cells function, but has no observable effect on suppressor cells number. One week post transfusion there was a fall in suppressor cells function that was followed by a marked increase in function by 2 weeks later. By 5 months post transfusion this rise in suppressor cell function had disappeared in majority of the patients. These findings were supported by Lenhard et al (1983). In contrast Jeannet et al(1982) reported that Con. A induced non specific suppressor cells are not triggered by blood transfusions.

A modest decrease in T_4 (helper/inducer T cells)/ T_8 (suppressor/cytotoxic T cells) and natural killer activity was reported as a part of the

normal immune response to repeated blood transfusions by Kaplan et al in 1984. Another study by Gascon et al (1984) shows depressed natural killer cell function but there was no significant decrease in T_4/T_8 ratio. Lenhard et al (1982) also showed that blood transfusions have no effect on T_4/T_8 ratio but there is a transient decrease in T cells count. However continuous increase of monocytes was noticed. These results were partially against the conclusion of Stiller et al (1981), who had suggested that impaired monocyte function or monocyte depletion following transfusion results in impaired cellular immunity. According to Lenhard et al (1982) post transfusion immunosuppressive activity is probably mediated by an unspecific monocytic suppressor cell.

Lenhard et al (1982) suggested thattwo different immune regulatory mechanisms play a part in post transfusion immunological abnormalities. In early post transfusion period, a non specific immunosuppression probably mediated by the action of monocytes and in the later phase increased suppressor cells activity may be responsible . Both effects are dependent on the number of transfusions and the time interval.

Keenan and Cascarpo (1979) have postulated that endocytosis of altered red cells impairs mononuclear phagocytic cell function resulting in supression of cell mediated responses.

There is evidence of changes in antibody response after blood transfusion with the development of specific unresponsiveness related to development of anti-idiotypic antibodies against particular T cell clones (Binz et al, 1977; Sucsivforea et al, 1982; Singhal et al, 1983 and Singhal et al, 1982) and development of Fc receptor blocking antibodies (Macleod et al, 1983).

In has also been postulated that the non-specific immunosuppressive effect of blood transfusions is due to iron and other product of erythrocyte breakdown. Ferritin can suppress the T cell responsiveness in mixed leucocyte cultures (Hatzmar et al, 1979) and in a study of transfused renal dialysis patients there was a inverse correlation between serum ferritin levels and the ratio of helper to suppressor T cells in the blood (Dupont et al, 1983).

The elevation of non specific lymphocyte inhibitory factors in plasma may account in part for the immunodepressive effect obsdrved after multiple transfusions (Shenton et al, 1979).

In recent years Waymack et al (1986) studied the effect of blood transfusion on traumatised rats. His observations suggest that the transfusion have no effect on the white blood cell counts, differential cell counts or neutrophil migration and bactericidal

index. Those animals that received transfusions did exhibit impaired cell mediated immunity and macrophage migration and this immunosuppressive effect of the blood transfusion may be due to, at least in part, by increasing macrophage suppression or lymphocyte response to stimuli.

MATERIAL AND METHODS

MATERIAL AND METHODS

Subjects for study were patients admitted in various surgical and non surgical wards of M.L.B. Medical College, Hospital, Jhansi(U.P.), India.

The patients were divided into two age and sex matched groups :

Group I : Surgical patients.

Group II : Non surgical patients.

The surgical patients were then separated into two subgroups :

a. Patients undergoing surgery without transfusion (Group Ia).

b. Patients receiving blood transfusion during surgery (Group Ib).

Patients with malignant diseases were not considered.

The non surgical group included age and sex matched patients with minor medical problems who received blood transfusion.

TESTS PERFORMED

1. Total leucocyte count and differential leucocyte count (Dacie and Lewis, 1974).
2. Absolute lymphocyte count (ALC).
3. Percentage and absolute T lymphocyte count(T % ALC). (E rosette) (Fudenberg et al, 1973).
4. Intra dermal PHA skin test (Blaese et al, 1973).

BLOOD COLLECTION

10 ml of venous blood from the anticubital vein of the patient was drawn by an autoclaved all glass syringe and poured in a sterilized glass test tube containing 25 IU heparin /ml. for T lymphocyte count. After gentle mixing the test tube was kept at room temperature in a vertical position for an hour to allow red blood cells to sediment. 2 ml of blood was also collected in a double oxalate vial for total and differential leucocyte count. Samples were taken pretransfusion/surgery, 1st day after transfusion/ surgery and 7th day posttransfusion/surgery.

INVESTIGATIONS

1. Total and Differential Leucocyte Count

It was carried out by standard technique as described by Dacie and Lewis (1974).

2. Absolute Lymphocyte Count

It was calculated by the formula :

$$ALC = \frac{TLC \times \text{Percentage of Lymphocyte}}{100}$$

3. T Lymphocyte Count : (E rosette).

MATERIAL

a. Heparin (5000 I.U./ml).

b. 20 ml. all glass syringe and 20 gauge hypodermic needle.

c. Calibrated centrifuge tube and plain glass test tube.

d. Pasteur pipettes (20 cm long).

e. Stop watch.

f. Sterilised isotonic saline.

g. Centrifuge machine calibrated for 100 to 500 g centrifuge forces.

h. One percent Trypan blue in normal saline.

i. Haemocytometer.

j. Light microscope.

METHODS

I. Sheep's Red Blood Cells Suspension :

Venous blood from anterior jugular vein of a healthy sheep was collected in a heparinised bottle (25 IU per ml). The bottle was shaken gently for proper mixing to prevent clotting. Blood was stored at 4°C to 6°C for a maximum period of two weeks. Blood from the same sheep was used throughout the study. Heparinised RBC were washed thrice in normal saline and centrifuged at 500 g for 5 minutes each time. Supernatant was discarded and finally a two percent suspension of cells was made in normal saline. This suspension was used for two weeks unless haemolysed.

II. Preparation of Lymphocyte Suspension

Ten ml of whole heparinised blood was collected from the patient and allowed to stand vertically for one hour to sediment red blood cells. the supernatant leucocyte rich plasma was taken with a pasteur pipette and centrifuged at 200 g for 5 minutes (approximately 1000 r.p.m.). The sediment was washed

twice with normal saline after discarding the supernatant. The cells were finally resuspended in 2 ml of normal saline. The number of lymphocytes per cu mm in this suspension were counted in a Neubauer Counting chamber and a concentration of 2 x 10^6 cells per ml was adjusted with normal saline. Vitality of the cells was checked by adding one percent trypan blue to a drop of the cell suspension on a slice. Vital cells excluded the dye.

III. Examination and Counting of the Percentage of T lymphocyte by E rosette Formation (Fudenberg et al, 1975).

a. 0.25 ml of a 2 percent SRBC was mixed with 0.25 ml of lymphocyte suspension and incubated at 37°C for 10 minutes after a thorough mixing. It was centrifuged at 100 g for 5 minutes and then kept at 4°C for one and half hours to four hours (average 2 hours).

b. An improved Neubauer chamber was washed, cleaned, dried and kept at 4°C for 10 minutes. The top layer cells of SRBC lymphocyte mixture was gently agitated and a small drop of this was placed on the chilled Neubauer chamber with a pasteur pipette and a coverslip was placed on it with great care. The chamber was left on the microscope undisturbed for 30 seconds to allow the cells to

settle. The number of lymphocytes forming rosette in 200 lymphocytes were counted and the percentage of T lymphocyte calculated. Lymphocytes with 3 or more adherent SRBC on the surface were considered as rosettes. From the T lymphocyte percentage the absolute values were calculated as follows :

$$\text{Absolute T Lymphocyte count} = \frac{\text{Percentage of T lymphocyte} \times \text{Absolute lymphocyte count}}{100}$$

IV. Intradermal Phytohaemagglutinin Test (Bloom et al, 1973).

Material

1. Phytohaemagglutinin (immunogen derived from phaseolus vulgaris).
2. Phosphate buffered saline.
3. Tuberculin syrine.
4. Pasteur pipette.
5. Occlusive dressing.

Method

Phytohaemagglutinin was used in a concentration of 10 ug/0.1 ml, using phosphate buffered saline for dilution. PHA was kept in 1 ml glass vials and kept frozen until just prior to use. It was given intradermally in a dose of 0.1 ml with a 26 no. needle. Induration was recorded at 24 hours to 48 hours using the method of Sokal et al (1973). The average diameter

of induration was calculated by taking the mean of the diameters in two perpendicular direction.

The pretransfusion/pre-operative test was done two days prior to surgery or transfusion. The post transfusion/post-operative tests were done on 7th and 14th days after transfusion or surgery.

OBSERVATIONS

OBSERVATIONS

The present study was done in our institute, M.L.B. Medical College, Hospital, Jhansi, between July, 1987 and July, 1988. During the period we studied the serial immunological parameters in 70 patients. Out of these, 10 patients underwent surgery without any transfusion (Group Ia), 45 patients received transfusion during surgery (Group Ib), while 15 patients received blood transfusions while being treated for medical disease (Group II).

The patients undergoing surgery without transfusion were those, who were operated for surgical procedures as vesical stone, renal stones, hernias, or benign prostatic hyperplasias.

The patients receiving blood transfusion during surgery were those, who were operated for benign prostatic hyperplasia, renal stones or benign gall bladder diseases and per-operative and post-operative period was without any complication.

The patients who received blood transfusion without any surgery were those, who received blood transfusion for some medical reason as severe anaemia or haemophilia.

All tests were done by one person under identical conditions.

A. LYMPHOCYTE COUNTS : (Lymphocyte percent - L% and Absolute lymphocyte count - ALC)

1. Group Ia

Lymphocyte percentage and ALC decreased in first 24 hours with recovery by seven days but the changes were statistically insignificant (P >0.2) (Table I, II).

2. Group Ib

The patients who received blood transfusion during surgery shows a marked decrease in lymphocyte percentage (L%) in first 24 hours i.e. from 31.4±13 to 21±11.6 percent (P <0.001). These patients showed a persistent decrease of L% (22±7.5) even after seven days (P <0.001). The absolute lymphocyte count was decreased markedly in first 24 hours, i.e. from 2587±1080 to 1732± 474 (P <0.001) with recovery at seventh day but the recovery was not complete i.e. - 2195±732 (P < 0.05) (Table I, II).

3. Group II

The lymphocyte percentage and absolute lymphocyte count were decreased in first 24 hours i.e. from 37.3±13 to 27.3±11.3 percent and from 2137±696 to 1504±342/mm^3, the difference being statistically significant (P <0.05 and <0.02). These lymphocyte counts tend to recover on seventh day (P >0.1 and >0.2) (Table I, II).

2. T-LYMPHOCYTE COUNTS (T-lymphocyte percentage - TM and Absolute T lymphocyte count - ATC).

1. Group Ia

The pre-operative values of T cell % and ATC was 64±4.2 percent and 1766±386 respectively and 24 hours after these values decreased to 48±6.3 and 1130±398 (p $<$0.001 and $<$0.005).

These values show a return towards normal on 7th post-operative day. The value, of TM on seventh post operative day was 59±6.8% (P $>$0.05) and of ATC was 1321±426 (P $<$0.05) (Table XII, IV).

2. Group Ib

Patients who received transfusion during surgery showed a marked decrease in T cell percentage i.e. from 56.6±3.4 to 33±9.3 (P $<$0.001) and ATC i.e. from 1482±679 to 369±202 (P $<$0.001). 24 hours after transfusion these values of TM and ATC remain significantly low(P $<$0.001) even on 7th post transfusion day (Table III, IV).

3. Group II

The TM and ATC decreased significantly 24 hours after transfusion (P $<$0.001) which increases towards pre-transfusion level on 7th day but remained significantly lower than the pretransfusion level (P $<$0.001) (Table III, IV).

C. PHA Skin Reactivity

The patients who underwent surgery without transfusion were unable to show any statistically significant difference from the pre-transfusion reactivity on 7th and 14th post-operative day ($P > 0.2$ and > 0.8). The patients who underwent surgery with transfusion showed a marked decrease in skin reactivity at seventh post-transfusion day ($P < 0.001$) which remained less than the pre-transfusion value even on 14th post-transfusion day ($P < 0.001$).

The non surgical patients with transfusion showed similar depressed skin reactivity to PHA at seventh ($P < 0.02$) and 14th ($P < 0.05$) post-transfusion day (Table V).

Table I : MEAN VALUES OF LA IN TRANSFUSED PATIENTS.

Groups	Subgroups	No.of cases	P.O.	P.O. 1	P.O. 7
I. Surgical	a. Without transfusion	10	30.0±3.6	24.0±10.6 N.S.	28.0±13.1 N.S.
	b. With transfusion	45	31.4±13.0	21.0±11.6 P $<$ 0.001	22.0±7.5 P $<$ 0.001
II. Nonsurgical	With transfusion	15	37.2±13.0	27.2±11.3 P $<$ 0.05	30.6±12.0 N.S.

P.O. = Pre operative/pre-transfusion

P.O.1 = 1st post-operative/post-transfusion day.

P.O.7 = 7th post-operative/post-transfusion day.

'p' values was calculated in comparison to pre-operative/pre-transfusion values.

Table II : MEAN VALUES OF ALC/mm^3 IN TRANSFUSED PATIENTS.

Groups	Subgroups	No.of cases	P.O.	P.O. 1	P.O. 7
I. Surgical	a. Without transfusion	10	2760±968	2355±1015 N.S.	2240±1463 N.S.
	b. With transfusion	45	2587±1008	1732±474 P $<$ 0.001	2195±732 P $<$ 0.05
II. Nonsurgical	With transfusion	15	2137±696	1504±342 P $<$ 0.02	1938±503 N.S.

P.O. = Pre operative/pre-transfusion

P.O.1= 1st post-operative/post transfusion day.

P.O. 7 = 7th post-operative/post-transfusion day.

ALC = Absolute lymphocyte count/mm^3.

'P' value was calculated in comparison to pre-operative/pre-transfusion values.

TABLE III : MEAN VALUES OF T-CELLS % IN TRANSFUSED PATIENTS.

Groups	Subgroups	No.of cases	P.O.	P.O. 1	P.O. 7
I. Surgical	a. Without transfusion	10	64.0±4.20	48.0±6.30 P <0.001	59.0±6.8 P >0.05
	b. With transfusion	45	36.6±3.40	33.0±9.50 P <0.001	40.3±5.80 P <0.001
II. Nonsurgical	With transfusion	15	39.3±2.35	42.2±2.87 P <0.001	46.3±3.3 P <0.001

P.O. = Pre operative/pre-transfusion.

P.O. 1 = 1st post-operative-/post-transfusion day.

P.O. 7 = 7th post-operative/post-transfusion day.

'P' value was calculated in comparison to pre-operative/pre-transfusion values.

TABLE IV : MEAN VALUES OF AIC/mm^3 IN TRANSFUSED PATIENTS.

Groups	Subgroups	No. of cases	P.O.	P.O. 1	P.O. 7
I. Surgical	a. Without transfusion	10	1766±386	1130±398 p = 0.005	1321±426 p < 0.05
	b. With transfusion	45	1482±678	569±202 p < 0.001	832±216 p < 0.001
II. Nonsurgical	With transfusion	15	1262±340	634±425 p < 0.001	897±231 p < 0.001

AIC = Absolute T lymphocyte count/mm^3

P.O. = Pre-operative/pre-transfusion.

P.O.1 = 1st post-operative/post-transfusion day.

P.O.7 = 7th post-operative/post-transfusion day.

'p' value was calculated in comparison to pre-operative/pre-transfusion values.

TABLE V : MEAN VALUES OF PHA SKIN REACTIVITY IN TRANSFUSED PATIENTS.

Groups	Subgroups	No.of cases	P.O.	P.O. 7	P.O. 14
I. Surgical	a. Without transfusion	10	24.2±1.3	23.8±1.4 N.S.	24.1±1.6 N.S.
	b. With transfusion	45	24.2±1.6	22.1±1.8 P $<$ 0.001	22.6±1.3 P $<$ 0.001
II. Nonsurgical	With transfusion	15	24.8±1.4	23.1±2.1 P $<$ 0.02	23.4±1.9 P $<$ 0.05

P.O. = Pre-operative/pre-transfusion

P.O. 7 = 7th post-operative/posttransfusion day.

P.O.14 = 14th post-operative/post-transfusion day.

'P' value was calculated in comparison to pre-operative/pre-transfusion values.

DISCUSSION

DISCUSSION

Lymphocyte and T-cells count are affected by number of variables. The lymphocyte count is subject to wide variations with viral diseases (Watkins et al, 1970), chronic illness and chronic malnutrition (Chandra, 1974) while there is no significant variation with age or sex (Kasharski et al, 1971 and Wakslar et al, 1974). The cause of variation in T-cell counts include the technique (Bach et al, 1969), source of sheep erythrocyte (Evans et al, 1973), concentration of heparin (Hadfield et al, 1975) and incubation period. The consensus of opinion is that incubation at $4^{\circ}C$ for one and half hour is the best period of testing T-cell count. To minimise the effect of the variables we used the same technique, equal periods of incubation, equal concentration of heparin and erythrocytes from the same sheep throughout the study. There are conflicting reports about the effect of age and sex on T-cells count (Carosella, 1974 and Mias Jouanen, 1975). Other factors which influence the T-cell count are smoking and alcohol intake (Landy, 1975) Marijuana smoking (Gupta et al, 1974) and corticosteroid treatment (Magnuson et al, 1976) which all caused a decreased T lymphocyte count in peripheral blood. Surgical stress leads to a transient fall in lymphocyte count and T cell count, which recover within two days (Slade et al, 1975). The time duration of the observed lymphocytopenia with rapid recovery by

24 to 48 hours corresponds to the period of maximal adrenal cortical secretion, suggesting that endogenous adrenal corticosteroid secretion is responsible in part for the results.

Impaired cell mediated immunity following transfusions has been observed by several authors (Fischer et al, 1980; Lenhard et al, 1982 and Kerman et al, 1982).

Our finding of a decrease in lymphocyte count in the post transfusion phase does not agree with Waymack et al (1986) who were not able to find any effect on the white blood cells counts or differential cell count neutrophil migration or bactericidal index. But they did show an impaired cell mediated immunity and macrophage migration. The post transfusion depression seen by us in lymphocyte count in both surgical and non surgical patients obviously cannot be explained only by surgical stress. Surgical stress leads to a fall in lymphocyte count which recovers with in two days (Slade et al, 1975). Our finding of persistent depressed lymphocyte count with a trend towards reversal after 7 days in surgical patients with transfusion and transient but significant depression of lymphocyte count in first 24 hours after transfusion with recovery within seven days in patients who received transfusion without surgery are similar to the findings of Schechter et al (1972). Schechter et al (1972) claim that blood transfusion as an

immunologic stimulant, the result was based on increase in activated lymphocyte identified by atypical lymphocyte or ^{3}H thymidine incorporation. Although it is possible that activated lymphocytes and their products modulate natural killer cells activity (Richardi et al, 1982) and may be responsible for post transfusion immunodepression.

Similarly the changes seen in T-cells count can only be attributed to immunodepressive effect because post operatively depressed T-cell counts due to surgical stress return back to normal within 48 hours (Slade et al, 1975). In our study a highly significant depression was seen to persist even at seven days. In this context our findings agree with those of Lenhard et al (1982) and Kerman et al (1982). But do not agree with Smith et al (1981) who were unable to show any change in no of T suppressor cells. Kaplan et al (1984) showed a decrease in OKT4/OKT8 (helper/suppressor) T lymphocyte ratios and a decreased natural killer cell activity in patients receiving repeated blood transfusions. Lenhard et al (1982) showed a transient decrease in T-cells in the post transfusion period after 3 units of blood transfusion. They, however, did not find any change in the number of helper/inducer cells or suppressor/cytotoxic effector cells. Monocytes showed a continuous increase. Smith et al (1981) reported that three weeks after transfusion there was

a significant increase of suppressor T-cell function in dialysis patients. A correlation between number of transfusions and cellular immune reactivity was established by Watson et al (1979) with greater depression after multiple transfusions. Kerman et al (1982 and 1983) showed a decrease percentage of Active T-rosette forming cells as well as increased percent of OKT8 +T cells. These changes were transient and resolution usually occurred within 14 to 21 days to pre transfusion level. This pattern of change was repeated with each blood transfusion leading to a stepwise depression of immune responsiveness with increasing number of blood transfusions, causing a more durable decrease in A-TRFC or spontaneous blasto-genesis and increase in OKT'8+cells. Smith et al (1981) showed that a single transfusion of two units of packed red blood cells in renal dialysis patient produces a significant effect on suppressor cells function but has no observable effect on suppressor cells number. One week after transfusion there was a fall in suppressor cell function that was followed by a marked increase in function two weeks later. By five months post transfusion, this rise in suppressor cells function had disappeared in the majority of patients. Additional evidence was provided by Cosson et al (1984) who showed evidence of decreased natural killer cell's activity. According to them transfused

patients not only have evidence of decreased cell mediated immunological capabilities but also of chronic immunological stimulation as shown by increased T-cell HLA-DR expression. Kapadia et al (1980) and Ballas et al, (1980) showed abnormal immunoglobin levels in post erythrocyte transfusion phase. Munn et al (1981) showed decreased in vitro T-cell responses to foreign antigen after blood transfusion.

Apart from the quantitative changes a number of qualitative changes were also elucidated in the post transfusion phase. Lenhard et al (1982) showed that mononuclear cells of transfused patients suppress the lymphocyte response to antigen as well as mixed lymphocyte reaction (MLR). Further in 1983 Lehhard et al reported on a series of patients with renal failure receiving pretransplant blood transfusion who were evaluated immunologically before and serially after transfusion. The tests included lymphocyte response to stimulation by concanavalin A and to a combination antigen cocktail (AgC, Behringwerke Marburg) containing PPD, tetanus toxoid, streptolysin mumps and vaccinia antigen). In addition, mixed lymphocyte reaction and suppressor cell cultures were obtained. These tests disclosed a marked demurance in lymphocyte responsiveness to antigen stimulation to 54% of pretransfusion, within one week after transfusion. This was followed by a gradual return to normal after four weeks. A

second transfusion resulted in an even greater inhibition in lymphocyte responsiveness, with a full return to normal function not been achieved until six weeks later. The mixed lymphocyte reaction cultures disclosed increase suppressor cell activity two to four weeks following transfusion with return to normal function at 12 weeks. Similar results were reported by Fischer et al (1980). Fortmann and Bingten (1982) showed identical low mixed lymphocyte reaction and low PHA responses in post multiple transfused patient. In contrast Varghese et al (1981) found normal PHA response of lymphocyte from thalassemic patients given repeated blood transfusions. Berleffs and Marquet (1981) have also shown depressed PHA response after transfusion in rhesus monkeys.

The depressed PHA skin response had not been documented to date in the post transfusion phase. In our study decreased PHA skin reactivity in transfused patients with or without surgery persisted even after 14 days and may correspond to the decrease in lymphocyte responsiveness to antigen stimulation in one week of transfusion and returning back to normal after four week as seen by Lenhard et al (1983). Our findings also agree with those of Fischer et al (1980) and Fortmann and Bingten (1982).

Waymack et al (1986) reported that blood transfusion did adversely effect macrophage function.

The animals received the transfusion had a 73% decrease in macrophage migration into the peritoneal cavity in response to a chemical peritonitis.

The exact cause of the immunodepression is not known but it has been variously described to be a part of the normal immune response to chronic allo-antigenic stimulation as shown by increased T cell HLA-DR expression (Gascon et al, 1984 and Kaplan et al, 1984).

Subjects acutely infected with Epstein Barr Virus (EBV) and cyto megalo virus (CMV) usually have low helper/suppressor ratios, accompanied by increase in relative and absolute number of suppressor cells with only a relative reduction in helper cells (Reinherz et al, 1980). EBV and CMV are common blood borne viruses and can cause post transfusion reduction in T-helper/T. suppressor cells ratio. However, the viral infection can not decrease the natural killer cells activity which is also a part of the post transfusion immunodepression. Ricardi et al (1982) suggest that it is possible that activated lymphocytes and their products modulate natural killer cell activity.

Lenhard et al (1985) showed that transfusion induce release of prostaglandins, activate suppressor T cells. Wynmuck et al (1986) suggest that if the

macrophages are unable to migrate to inflammatory site, they would be unable to accomplish their part of the cell mediated immune (CMI) response. Further the transfusion may alter the secretion of lymphokines by the macrophages. Waymack et al,(1986) documented an increased production of the immunosuppressor metabolite prostaglandin by macrophages, isolated from transfused rats who had sustained injury. Prostaglandin E inhibits the lymphocyte function (Goldyne et al, 1981 and Goodwin et al, 1980). Waymack et al (1986), suggest that important contributory factors for immunodepressive effect of blood transfusions are not related to histo-compatibility. The factors could include haemolysis and lysis of platelets and/or neutrophils. Keown and Deschamps (1979) have suggested that damaged red cells present in transfused blood may impair mononuclear phagocytic cell function resulting in suppression of immune responses. Lambard et al, (1982) observed significant increase of monocytes and Ia (Ia) positive cells in post transfusion period and concluded that unspecific monocytic suppressor cells are responsible for post transfusion immunosuppression.

Dupont et al, (1983) showed serum ferritin acts as a significant parameter associated with modification of the OKT4/OKT8 ratio. There is a well established association between ferritin and blood transfusion

(Cohal et al, 1979). Several studies demonstrate influence of iron or protein binding iron on immune response or markers. Iron modifies the traffic and distribution of lymphoid cells (de Sousa, 1978). Iron salts and saturated lactoferin, block, in vitro, active and late rosettes (Nishiya et al, 1980). So the raised serum ferritin level following multiple transfusion may be responsible for post transfusion immunodepression.

Jung et al, (1987) showed that infusion of paltelets, leads to a two fold immunosuppression, specific and non specific. Singhal et al, (1982) suggest that blood transfusions may induce anti-idiotypic antibodies and these antibodies are responsible for the imbalance of host's immunoregulatory circuit. This immunological unresponsiveness may be due to anti-idiotypic antibodies directed against T-cell receptors. This view is further supported by Pagnilli et al, (1982). Further cold B cell antibodies (Garner-Favre et al, 1980), immune complexes (Bonsonana et al, 1981) and Fc - receptor blocking antibodies (Haaland et al, 1983) may also play a part in post transfusion immunosuppression.

It would thus appear from our observations, that there is a depression of the cellular mediated immune response of the body secondary to blood transfusions. Although there is a fall in the T-cell count for upto 7 days the sub sets were not studied. But from the literature we find there is an increased suppressor cells activity and a decreased natural killer cells

activity. The depression of CMI is also corroborated by the depressed PHA response at 7th day. But the depression of the PHA skin response even at 14th day with a near normal T cells and lymphocyte count can only mean that the functional capacity of these cells have not yet returned to normal although the number has.

CONCLUSION

CONCLUSIONS

In the present study 70 patients were investigated serially to see the change in immunological parameters as a result of blood transfusions. We studied lymphocyte percentage, absolute lymphocyte count, T cell percentage, absolute T cell count and delayed hypersensitivity reaction to the antigen PHA. Out of these seventy patients, 10 patients underwent surgery without any transfusion, 45 patients received transfusion during surgery and 15 patients received transfusion without surgery.

The conclusions derived were as follows :

1. Surgery causes a transient fall in immunological parameters and hence fall in cell mediated immunity. which returns back to normal within 24to 48 hours.
2. Transfusion in patients undergoing surgery caused a depression of lymphocyte count and T-lymphocytes count for upto seven days with subsequent reversal towards normal and depression of PHA skin response persisting even after 14 days.
3. Transfusion in medical patients caused an identical cellular immune profile change as in surgical patients receiving transfusion.

Thus the present study shows that blood transfusion in patients causes significant but transient

fall in cell mediated immunity. The maximum depression occurring with in 24 hours with a phase of recovery of 3 to 4 weeks.

The cause and mechanism of the transfusion induced immunosuppression cannot be established by the present study and requires further work.

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